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EFFECT OF DIFFERENT CONCENTRATIONS OF 2, 4-D AND BAP ON CALLOGENESIS IN SUGARCANE CLONES

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ABSTRACT

Studies were carried out to establish an efficient system for callus induction in two sugarcane clones (2008T42 and 2009T5) during 2012. Leaf rolls were used as explants for callus induction. Two growth regulators, 2, 4-D and BAP with different concentrations (1-5 mg l^{-1}) were used for callus induction. Cultures were placed in 24 hours dark at 24 ± 2°C with 70% relative humidity. The two sugarcane clones (2008T42 and 2009T5) exhibited better response for callus induction in 2, 4-D at 4 mg l^{-1} with minimum number of days taken for callus initiation (9.7 - 10.2), maximum number of explants inducing callus (6.00) and the highest callus induction frequency (100 per cent).

KEY WORDS: BAP, Callus induction, 2, 4-D, Leaf roll

INTRODUCTION

Sugarcane (*Saccharum officinarum*) is one of the most important cash crops in India majorly used to produce cheap food in the form of sugar and gur that further lends itself for energy production. In order to meet the demands there is a need to increase cane productivity as expansion of area may not be possible. Sugarcane succumbs to decline because of its clonal propagation and increased incidence of diseases and pests over years. One of the major reason for low productivity is lack of quality seed material. Plant regeneration through tissue culture technique would be a better alternative for improving the quality of seed thereby production. Callus production is an essential step in the use of tissue culture studies for various physiological phenomena inducing resistance against various biotic and abiotic stresses. Callus is an unorganized, proliferative mass of predominantly parenchyma cells. The pioneering work on *in vitro* studies in sugarcane was conducted by Nickell (1964) who established first sugarcane callus cultures from mature internodal parenchyma tissue. Heinz *et al*. (1977) reported callus formation from parenchyma tissue of shoot apex and leaves of *Saccharum* spp. on MS (Murashige and Skoog, 1962) medium containing coconut water (10 per cent) and 2, 4-D.

Studies have suggested that amongst all the media tested for callus induction and proliferation by different workers, the best medium was modified MS medium (Liu and Chen, 1974; Guiderdoni, 1986; Aftab *et al*., 1996 and Baksha *et al*., 2002). Role of auxins have also been studied for callus induction and proliferation. Nadar *et al*. (1978) found that embryogenic callus forms when auxin is added to the medium. On the other hand, no embryogenesis was observed in callus cultures on auxin-free media. Callus proliferation in modified MS medium with various levels of auxins and cytokinins in sugarcane was also reported by Bhansali and Singh (1982) and Zang *et al*. (1983). Studies have shown that amongst different auxins tested for callus induction, addition of 2, 4-D in the medium always produced better callus growth than any other growth regulator. Kulkarni (1989) reported that callus induction and proliferation from immature sugarcane leaves triggers on medium containing 2, 4-D. Karim *et al*. (2002) while working on two sugarcane varieties, (Isd-16 and Isd-28) observed that the highest percentage of callus induction was obtained on MS basal medium supplemented with 3.0 mg l^{-1} 2, 4-D and 10 per cent coconut milk. Similarly, Mamun *et al*. (2004) also found that among all the tested auxins (IAA, 2, 4-D, IBA, and NAA), the best performance for callus induction was obtained on 3.0 mg l^{-1} 2, 4-D. The present study was taken up to know the effect of different concentrations of 2, 4-D along with BAP on callogenesis in sugarcane clones.

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MATERIAL AND METHODS

Pre-release sugarcane clones, 2008T42 (mid late) and 2009T5 (early) were chosen for the present investigation during 2012 at ARS, Perumallapalli. The plants were raised and maintained under field conditions as per the recommended agronomic practices. These plants served as the source of explants for all the *in vitro* studies conducted during the course of investigation.

Disease free and young sugarcane tops of 2008T42 and 2009T5 were selected from the top portion of plant. Spindle from the top was excised after removing young leaves. The collected explants were partially trimmed off and treated with 0.5 g l^{-1} ascorbic acid and 1.0 g l^{-1} PVP (Poly vinyl pyrrolidone) mixture followed by washing with liquid detergent water containing a few drops of Tween-20 (20 ml l^{-1}) for 5-10 minutes. After that explants were kept in an aqueous mixture solution of bavistin (10 g l^{-1}) and streptomycin (1 g l^{-1}) for 10-15 minutes. These explants were washed with sterile deionizing water for 3-5 washings, each for 3-5 minutes. Sterilization was done first with sodium hypochlorite (3.0%) for approximately 10 minutes. They were then sterilized with ethyl alcohol (70%) for 30 seconds and washed with sterile distilled water for 2-3 times. These last two steps were performed inside a laminar flow hood to maintain the aseptic condition of the explant and to prevent the reintroduction of contaminating microbes.

The cultures were incubated in a culture room maintained at a temperature of 25 ± 2°C, relative humidity of 70 per cent and 16 hours of photoperiod with light intensity of 2500 lux. Subcultures were done every 2-3 weeks according to the need of the experiment. Different concentrations of 2, 4-D and BAP were tried individually such as 2, 4-D (1.0, 2.0, 3.0, 4.0 and 5.0 mg l^{-1}) and BAP (1.0, 2.0, 3.0, 4.0 and 5.0 mg l^{-1}) with MS basal medium for callus induction.

RESULTS AND DISCUSSION

Among different concentrations of 2, 4-D and BAP, mean number of explants that induced callus ranged from 0.33 to 6.00 (Table 1). Maximum number of explants (6.00) that induced callus was recorded with 4 mg l^{-1} of 2, 4-D (T_4 and T_9) and minimum number of explants (0.33) that induced callus was recorded with 1 mg l^{-1} of BAP (T_{11} and T_{16}) in both sugarcane clones, 2008T42 and 2009T5. Callus with respect to 2, 4-D, maximum number of explants that induced callus was observed with 4 mg l^{-1} (T_4 and T_9) in both 2008T42 and 2009T5 followed by 3 mg l^{-1} in 2009T5 (T_8) and minimum in 1 mg l^{-1} (T_1 and T_6) for two sugarcane clones. Whereas in case of BAP 5 mg l^{-1} (T_{15} and T_{20}) had recorded maximum number of explants that induced callus followed by 4 mg l^{-1} (T_{19}) Minimum number of explants induced callus in both 2008T42 and 2009T5 with 1 mg l^{-1} (T_{11} and T_{16}). The clone, 2009T5 exhibited better response for mean number of explants inducing callus in different concentrations of 2, 4-D. whereas 2008T42, showed better response in BAP. In both growth hormones 2, 4-D was found to be better for callus induction in 2008T42 and 2009T5.

Mean number of days taken for callus initiation ranged from 9.7 to 31.8 (Table 1). Among all the treatments maximum number days (31.8) taken for callus initiation was recorded with 1 mg l^{-1} of BAP (T_{11}) in 2008T42 and minimum number days (9.7) was recorded with 4 mg l^{-1} 2, 4-D (T_4) in 2008T42. The concentration of 4 mg l^{-1} of 2, 4-D (T_4 and T_9) had recorded minimum number of days taken for callus initiation in both the clones followed by 3 mg l^{-1} (T_4) and 5 mg l^{-1} (T_5) in 2008T42. In contrast, both the clones took maximum number of days with 1 mg l^{-1} and 2 mg l^{-1}. With respect to BAP maximum number of days taken for callus initiation was recorded at 1 mg l^{-1} (T_{11} and T_{16}) in both sugarcane clones followed by 2 mg l^{-1}. Whereas, minimum number of days for callus initiation was recorded at 5 mg l^{-1} of BAP in both the clones. A minimum mean number of days taken for callus initiation was recorded in 2, 4-D treatments than BAP treatments. Within the concentrations of 2, 4-D, 2008T42 recorded minimum number of days than 2009T5. The clone, 2009T5 was found to have taken minimum number of days for callus initiation with BAP treatments when compared with 2008T42.

Callus induction frequency ranged from 5.50 to 100 percent in different concentrations of 2, 4-D and BAP (Table 1). Among all treatments, 1 mg l^{-1} of BAP showed poor response towards callus induction as it accounted only 5.50 percent and the highest response was recorded (100 per cent) with 4 mg l^{-1} of 2, 4-D in both sugarcane clones. In case of 2, 4-D the concentration of 4 mg l^{-1} had recorded 100 per cent callus induction in 2008T42 and 2009T5 and the lowest frequency was recorded in 2008T42 (55.56 percent) and in 2009T5 (61.06 percent) with 1 mg l^{-1} of 2, 4-D. Whereas in case of BAP, the concentration of 5 mg l^{-1} recorded the highest callus induction frequency of 49.39 per cent in 2008T42 and 50.33 per cent in 2009T5. The lowest frequency was

Table 1. Effect of different concentrations of 2, 4-D and BAP on callus formation in sugarcane clones

Treatments	Variety	MS media + Growth Hormones	Mean no. of explants induced callusing	Callus induction frequency (%)	Mean no. of days for callus initiation
T_1	2008T42	2, 4-D 1 mg l^{-1}	3.33	55.56 (48.17)	15.9
T_2	2008T42	2, 4-D 2 mg l^{-1}	3.83	63.89 (53.04)	13.9
T_3	2008T42	2, 4-D 3 mg l^{-1}	5.33	88.89 (70.51)	12.5
T_4	2008T42	2, 4-D 4 mg l^{-1}	6.00	100.00 (90.00)	9.7
T_5	2008T42	2, 4-D 5 mg l^{-1}	5.00	83.33 (65.87)	12.7
T_6	2009T5	2, 4-D 1 mg l^{-1}	3.66	61.06 (51.36)	16.0
T_7	2009T5	2, 4-D 2 mg l^{-1}	4.33	72.22 (58.17)	14.5
T_8	2009T5	2, 4-D 3 mg l^{-1}	5.66	94.39 (76.26)	12.6
T_9	2009T5	2, 4-D 4 mg l^{-1}	6.00	100.00 (90.00)	10.2
T_{10}	2009T5	2, 4-D 5 mg l^{-1}	5.21	88.89 (70.51)	13.2
T_{11}	2008T42	BAP 1 mg l^{-1}	0.33	5.50 (13.55)	31.8
T_{12}	2008T42	BAP 2 mg l^{-1}	0.66	11.00 (19.36)	30.2
T_{13}	2008T42	BAP 3 mg l^{-1}	1.60	26.78 (31.14)	29.0
T_{14}	2008T42	BAP 4 mg l^{-1}	2.08	34.72 (36.08)	26.9
T_{15}	2008T42	BAP 5 mg l^{-1}	3.14	49.39 (44.63)	23.4
T_{16}	2009T5	BAP 1 mg l^{-1}	0.33	5.50 (13.55)	31.2
T_{17}	2009T5	BAP 2 mg l^{-1}	0.66	11.00 (19.36)	30.2
T_{18}	2009T5	BAP 3 mg l^{-1}	1.95	32.61 (34.88)	26.9
T_{19}	2009T5	BAP 4 mg l^{-1}	2.15	35.83 (36.75)	24.5
T_{20}	2009T5	BAP 5 mg l^{-1}	3.02	50.33 (45.77)	21.7
C.D at 5%			0.061	0.751	0.751
(±) SE(m)			0.021	0.262	0.260

Values in parentheses represent arc sine transformed values

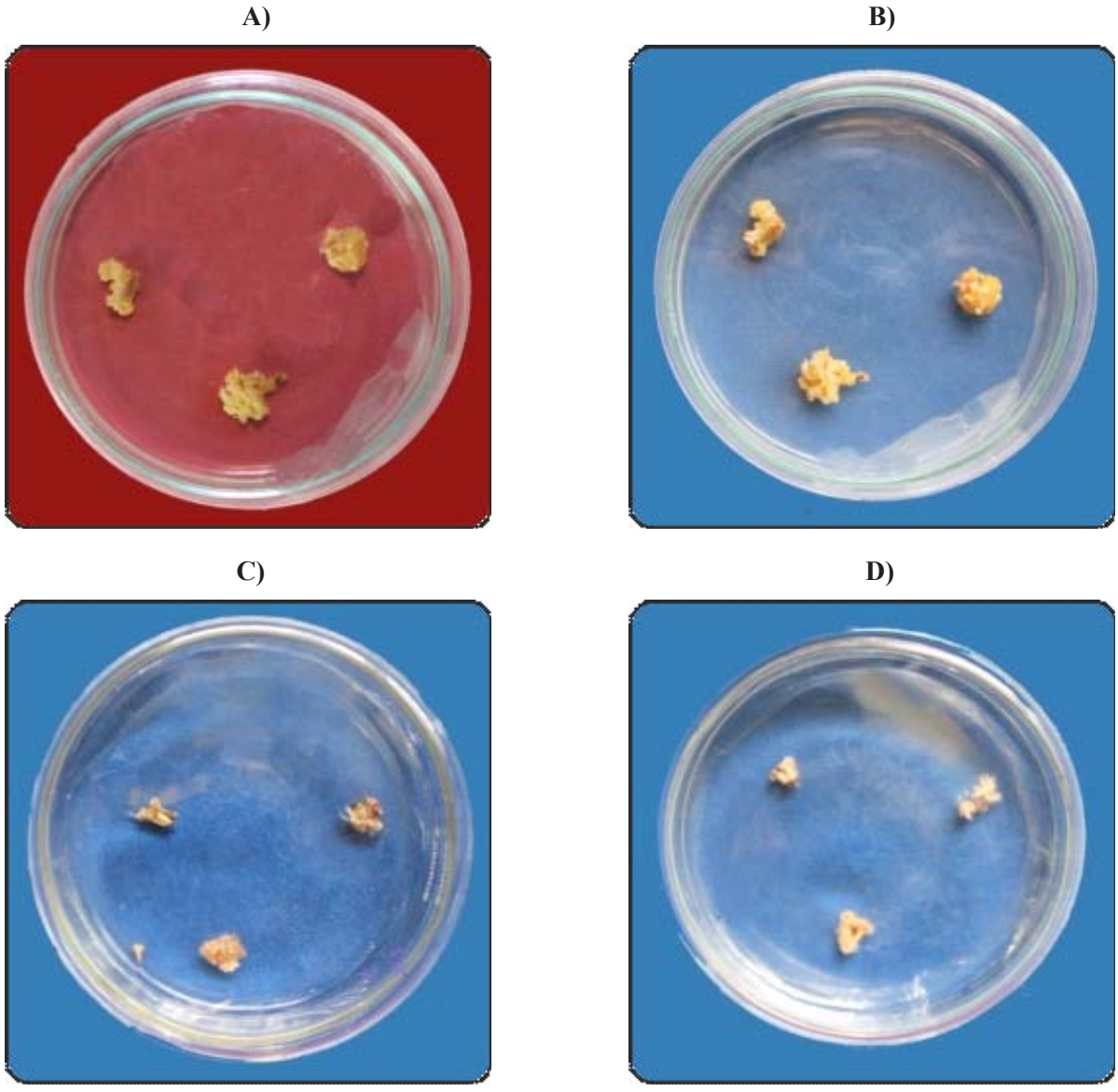

Fig. 1. A) Callus induction in 2008T42 at 4 mg l^1 2, 4-D; B) Callus induction in 2009T5 at 4 mg l^{-1} 2, 4-D; C) Callus induction in 2008T42 at 1 mg l^{-1} BAP; D) Callus induction in 2009T5 at 1 mg l^{-1} BAP

recorded as 5.50 per cent in both 2008T42 and 2009T5 at 1 mg l^{-1} of BAP. In both the clones, it was observed that callus induction frequency was maximum with 2, 4-D than BAP. The clone, 2009T5 exhibited superior response in both treatments of 2, 4-D and BAP than 2008T42.

The results revealed that the two sugarcane clones (2008T42 and 2009T5) exhibited better response with 2, 4-D than BAP in all hormonal treatments for callus induction with minimum number of days taken for callus initiation, maximum number of explants that induced callus and the highest callus induction frequency. Callus formation occurred in all the concentrations of 2, 4-D but in case of BAP it was poor in 1mg l^{-1} and 2 mg l^{-1} concentrations. The explants showed slight swelling at 1mg l^{-1} and 2 mg l^{-1} of BAP and subsequently dried. Thus it was observed that presence of auxin (2, 4-D) is essential for callus induction. Similar results were also reported by Islam *et al.* (1996) and Hossain *et al*. (1996). 2, 4-D was significantly effective in the initiation of callus for sugarcane clones. The development of callus from immature leaf roll explants is directly related to the presence of 2, 4-D which is a suitable growth hormone responsible for callus induction in most plant species in plant tissue culture work. This is similar to the findings

of Mamun *et al*. (2004) and Baksha *et al*. (2002). The production of callus at cut edge of explant may be due to the wound caused during the process of cutting which resulted in a synchronous cell division. This is a process of de-differentiation of organized tissue as was opined by Hamish and Sue (1989), Pellegrinechi *et al*. (2004), Qin *et al.* (2005) and Xing *et al*. (2010). Among all the treatments, 4 mg l^{-1} of 2, 4-D was proved to be the best for callus induction in sugarcane clones (Fig 1). The results are in agreement with results obtained by Chanwit (1994), Mannan and Amin (1999) and Tahir *et al*. (2011) on different genotypes of sugarcane. Callus induction was gradually enhanced with increase in concentrations of 2, 4-D. This observation was supported by Sani and Mustapha (2010), Qin *et al*. (2005) and Xing *et al*. (2010).

The results also revealed that there was slight genotypic difference in callus induction. The sugarcane clone 2009T5 showed better response than 2008T42 with different concentrations of the two hormones. Similar genotypic differences were also reported by Taylor et *al.* (1995) and Shahid *et al*. (2011). The number of days taken for callus induction decreased with increased concentrations of 2, 4-D and BAP. Number of explants initiating callus and callus induction frequency increased with increased concentration of 2, 4-D and BAP.

CONCLUSION

Callus induction as measured by minimum number of days taken for callus initiation (9.7 -10.2), maximum number of explants inducing callus (6.00) and the highest callus induction frequency (100 per cent) was higher with 4 mg l^{-1} of 2, 4-D than BAP in two sugarcane clones 2009T5 and 2008T42. The sugarcane clone 2009T5 showed better response than 2008T42 with different concentrations of the two hormones. Hence 4 mg l^{-1} of 2, 4-D can be considered as the basic requirement for callusing in sugarcane for proceeding further investigations like micropropagation and production of somaclonal variants.

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